UAB Symposium

Congenital Bone Marrow Failure
Path to Cure
Patient’s Journey

- 8 yo male
- Generally healthy
- Short stature (5%ile)
- Hypermobile thumbs with flattened thenar eminences
- Hair and nails normal
- 1 year history of low platelet counts
Referral to Hematologist

CBC worsened over the year

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Result</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>2.7 x 10^9/L</td>
<td>4.0-11.0</td>
</tr>
<tr>
<td>ANC</td>
<td>1.8 x 10^9/L</td>
<td>1.5-8.0</td>
</tr>
<tr>
<td>Hgb</td>
<td>13.8 g/dL</td>
<td>13.3-17.7</td>
</tr>
<tr>
<td>MCV</td>
<td>103 fL</td>
<td>78-100</td>
</tr>
<tr>
<td>Plt</td>
<td>54 x 10^9/L</td>
<td>150-450</td>
</tr>
</tbody>
</table>
Interconnected Pathways of Congenital BMF

Diamond Blackfan Anemia
Disorder of ribosomal proteins

Fanconi Anemia
Disorder of DNA repair

Dyskeratosis Congenita
Disorder of proteins that maintain telomers
Diagnostic Mutagen Stress Test
Classic Manifestations of Fanconi Anemia

Can effect every organ in the body

- Skin (café au lait) 71%
- Radial Ray 59%
- Kidney/urinary 57%
- Short stature 67%
- Ears/deafness ?
- CNS 1%
- Eyes 47%
- GI (esoph, duod) 7%
- Cardiac (PDA/VSD) 29%

‘Classic’ phenotype – missing thumb, missing kidney, short and bone marrow failure
Effect of Incorrect Diagnosis

- V – Vertebral fusions
- A – Anal atresia
- C – Cardiac (PDA and VSD)
- TE – Tracheo-Esophageal fistula
- R – Renal (hydronephrosis)/radial defects
- L – Limb (non radial) defects

Devastating Effects

- No surveillance for MDS/leukemia
- Incorrect reproductive counseling (or evaluation of existing siblings)
When to test for FA

- **Absolute indications for DEB testing**
  - aplastic anemia (any age)

- **Possible indications for DEB testing**
  - young patient with H/N cancer
  - MDS with complex cytogenetic abnormalities
  - constellation of macrocytic anemia, short stature
  - VATER/VACTERL (don’t be swayed by prior dx)
FA VATER Signature

Higher frequency of renal and limb (radial and/or thumb) anomalies

- 93% in FA VATER
- <30% in non FA VATER

Skewed distribution within FA

- FANCB (21%)
- FANCD1/BRCA2 (14%)
- FANCD2 (12%)
- FANCA (19%)

Recommendation

All VATER patients with renal and limb anomalies, then test for FA
General Pattern of Bone Marrow Failure

- SAA
  Aplastic Anemia
- MDS
  Myelodysplastic syndrome
- AML
  Acute myeloid leukemia

Blood counts vs. Time
- Viral infection
- Just recheck counts more frequently
Complex Cytogenetic Abnormality

Clone 1

ISCN: 46,XY,dup(1)(q12q32)[9]/46,XY,del(20)(q11.2q13.3)[10]/46,XY[1]

INTERPRETATION:
Two abnormal clones were identified. **Clone 1**, comprises 45% of metaphases; duplication within the long arm of chromosome 1, extending from band q12 to q32.

**Clone 2**, comprises the remaining 55% of metaphases, had a deletion within the long arm of chromosome 20, extending from band q11.2 to q13.3.
Impact of Overriding the G2/M Arrest

Clonal Chromosomal Abnormalities

Betsy Hirsch, Ph.D.
Evolution of Clonal Chromosomal Abnormalities

What do they mean?
More frequent marrow exams

Betsy Hirsch, Ph.D.
~16% do not have BMF, MDS or Acute Leukemia by age 18 years
Pathophysiology of BMF, MDS and Acute Leukemia in FA

Oligoclonal hematopoiesis
- marrow failure
- MDS / leukemia (evading G2/M arrest)

DNA damage

Viral suppression
[INFγ hypersensitivity]
The Fanconi Anemia Pathway of DNA Repair and Human Cancer
By Vaidehi Krishnan, Lavina Sierra Tay and Yoshiaki Ito

Unique issues
- impact on carriers
- genetic testing in children who are potential donors
Genotype / Phenotype Correlations
Time to MDS or Leukemia

- **FANCC**
  - n=78 FANCA+G
  - n=253
- **Other FANC Groups**
  - n=415

High risk of other malignancies before and after HSCT

- **FANCD1/BRCA2**
  - n=14
  - All <7 years of age

Cumulative Incidence

Time to Hematologic Malignancy (Months)
FANCC Genotype-Phenotype Correlations

Onset of Marrow Failure

Survival

Indications for HSC Transplant

• Severe cytopenia
  ○ Hgb <8 g/dL
  ○ ANC <500/mm³
  ○ PLT <20,000/mm³

• MDS/Leukemia
Eligibility Requirements

- Adequate organ function
  - Renal (GFR > 30)
  - Liver (ALT < 3 x normal)
  - Cardiac (EF > 45)

- Available donor
  - Sibling BM (without FA)
  - Unrelated BM
  - Unrelated UCB
  - Haplo BM
Conditioning Dilemma in FA

- TBI 450 rather than 1200 cGy
- Cyclophosphamide 40 rather than 200 mg/kg
- ATG

T cell depleted HSC

Principles:
- Myeloablation
  - Destroy Leukemia and the Host’s Immune System
- Immunosuppression
  - Prevention of HVG and GVH reactions

CSA
Matched Sibling Donor BMT for FA

Trial 1 - CY, ATG, TLI 450
Trial 2 - CY, ATG, FLU, TCD

Eliminated Radiation
TCD to reduce risk of GVHD
Eliminated MP

University of Minnesota
State of the Art
Sibling Donor Transplants after 2000

Complications are rare except in those with MDS/Leukemia or adult age.
Unrelated Donor BMT for FA

Trial 1 - CY, TBI 450
Trial 2 - CY, ATG*, TBI 450, TCD
Trial 3 - CY, ATG, FLU, TBI 450, TCD
Trial 4 - CY, ATG, FLU, TBI 450 + thymic shielding, TCD
Trial 5 - CY, ATG, FLU, TBI 300 + thymic shielding, TCD

Day -3 to 180
Until ANC 2500
Prior to HCT, CT is performed to locate the thymus

Thymus blocks attached to TBI stand brackets securing the lung compensators
Perserving Fertility
Transient Transposition of the Ovary
Survival in FA Patients

Unrelated Donors

- FLU CY TBI (300) + TS: 89%
- FLU CY TBI (450) + TS: 75%
- FLU CY TBI (450): 67%
- CY TBI (450): 23%
Androgens – negative effect in univariate analysis (trend in multivariate)
High Expectations for Survival after BMT

Figure 3. Probability of survival after HCT in patients without a prior history of opportunistic infection or transfusions who received conditioning with TBI 300 cGy and thymic shielding, CY, FLU, and ATG.
Alternatives to Transplant

No donor

Very poor organ function
- Kidney
- Liver
- Cardiac

Pre-existing infections
- Lung
- Blood

Older Patient Age
- ~45 (sibling donor)
- ~40 (unrelated donor)

Alternatives
- Androgens
- Intermittent use of blood growth factor and transfusions – other supportive care
- Experimental therapy (gene therapy or novel transplant protocol)
Cancer Risk

**Incidence 30% by age 45 years**

Observed to Expected Ratio

- Leukemia  785
- Head/Neck  706
- Esophagus  2362
- Liver      386
- Total      50

Does Transplant Alter the Risk

Conclusions

- Transplant is associated with high rate of solid tumors, particularly SCCs

- Association between GVHD [or its treatment] and SCCs
  - 33-fold higher risk with cGVHD

How do we reduce cancer risk?

- **Eliminate Bu and TBI**
  - Antibody based conditioning
  - Higher stem cell doses
  - PGD/IVF for HLA match and absence of FA

- **Prevent GVHD**
  - T cell depletion of the graft
  - Use of UCB
  - PGD/IVF for HLA match and absence of FA
  - Gene therapy

- **Enhance immune reconstitution**
  - More HSCs
  - Thymic progenitors Opportunistic infection
  - Thymic shielding
  - PGD/IVF for HLA match and absence of FA
  - Gene therapy
Low risk of Grade II-IV AGvHD with T Cell Depletion

Cumulative Incidence

Days Post-TX

Alternative donor (std. risk)

Matched sibling

P = 0.47
Low risk of Chronic GvHD with T Cell Depletion

P = 0.24
Directions to Reduce Cancer Risk in Fanconi Anemia

Risk by Patient Age

Cumulative Incidence

Years of Age

University of Minnesota

19% (7-31%) by age 45 years
StemRegenin-1
Aryl Hydrocarbon Receptor (AhR) Pathway

- SR-1 directly binds and inhibits the AhR, blocking induction of CYP1B1 and impeding stem cell differentiation
- SR-1 mediated massive UCB stem cell expansion

Boitano et al. Science 2010; 1345-1348
Massive Expansion of Cord Blood Stem Cells
327-fold (n=37)
MGTA-456 after TBI 1320 cGy
Faster Neutrophil Recovery, 100% engraftment

Historical 83% (CI, 74-91)
N=121
median 26.5 d (13-42)

MGTA-456 (single) 100%
N=9/9
median 15 days

p < 0.001
Accelerated Immune Reconstitution after UCBT

Myeloablative Conditioning

Absolute CD4 200 at 2-3 months
Future of SR-1 Expanded HSPCs
Manufacture of Thymic Progenitors

T Progenitor Dose Escalation
- 0.1 x 10^6 /kg
- 0.3 x 10^6 /kg
- 1.0 x 10^6 /kg
- 3.0 x 10^6 /kg
- 10.0 x 10^6 /kg

Ex vivo culture – HSC835

Re-Cryopreserved

CD34+
Selection

UCB 1 → CD34

CD34+ Selection

20% or maximum available

Tprog ex vivo culture

Repeat CD34+ Selection

T cell tracking

Days
Alcohol / Food Exposures and Cancer Risk
Savior Sibling
IVF / PGD

Use of assisted reproductive techniques

IVF

Preimplantation Genetic Diagnosis
PGD to prevent disease and save the life of an existing child

PGD for mutation

To eliminate risk of genetic disease

PGD for HLA

Savior Sibling

To guarantee an HLA identical HSC donor
The Candidates

1995

Molly

Henry
FANCC IVS4 A>T Mutation
Predicting the Future

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time to</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM Failure</td>
<td>4 years</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>6 years</td>
</tr>
<tr>
<td>Survival</td>
<td>10 years</td>
</tr>
</tbody>
</table>
Verlinsky et al. JAMA 2004; 285,3130
Pregnancy
The Creation of Adam
Back Lash

Brutum Fulmen:
OR THE
B U L L
Of Pope Pius V.

Q. ELIZABETH,
As with the
Abdication of her Subjects from their Oath of Allegiance, with a Peremptory Injunction, upon
Part of an Anathema, never to obey any
Of Her Laws or Commands.

With some Observations and Anecdotes annexed.

By THOMAS Lord Bishop of Lincoln.

Notwithstanding the Bull of Pope Paul the Third, containing the Damnation, Excommunication, &c.
of King Harry the Eighth.

Concerning his late Policy, how many of his Subjects

Printed by S. Royce, at the Sign of St. Paul's
in St. Paul's Churchyard. MD LXX.

[Image of St. Peter's Square in Rome]
The path of the ‘slippery slope’

Disease Prevention

HLA Match

Gender

Physical Features

Longevity

Slippery Slope
Designer Babies

Scientists say that, with gene therapy, they may soon be able to cure a child's inherited disease before he is even born. But should they be allowed to create kids with made-to-order traits? BY SHARON BEGLEY

IT IS ONLY A MATTER OF TIME. ONE day—a day probably no more distant than the first wedding anniversary of a couple who are now teenage sweethearts—a man and a woman will walk into an in vitro fertilization clinic and make scientific history. Their problem won't be infertility, the reason couples now choose IVF. Rather, they will be desperate for a very special child, a child who will elude a family curse. To create their dream child, doctors will fertilize a few of the woman's eggs with her husband's sperm, as IVF clinics do today. But then they will inject an artificial human chromosome, carrying made-to-order genes like pearls on a string, into the fertilized egg. One of the genes will carry instructions ordering cells to commit suicide (graphic). Then the doctors will place the embryo into the woman's uterus. If her baby is a boy, when he becomes an old man he, like his father and grandfather before him, will develop prostate cancer. But the cell-suicide gene will make his prostate cells self-destruct. The man, unlike his ancestors, will not die of the cancer. And since the gene that the doctors gave him copied itself into every cell of his body, including his sperm, his sons will beat prostate cancer, too.

Genetic engineers are preparing to cross what has long been an ethical Rubicon. Since 1990, gene therapy has meant slipping a healthy gene into the cells of one organ of a patient suffering from a genetic disease. Soon, it may mean something much more momentous: altering a fertilized egg so that genes in all of a person's cells, including eggs or sperm, also carry a gene that scientists, not parents, bequeathed them. When the pioneers of gene therapy first requested government approval for their experiments in 1987, they vowed they would never alter patients' eggs or sperm. That was then. This is now. One of those pioneers, Dr. W. French Anderson of the University of Southern California, recently put the National Institutes of Health on notice. Within two or three years, he said, he would ask approval to use gene therapy on a fetus that has been diagnosed with a deadly inherited disease. The therapy would cure the fetus before it is born. But the introduced genes, though targeted at only blood or immune-system cells, might inadvertently slip into the child's egg (or sperm) cells, too. If that happens, the genetic change would affect that child's children unto the nth generation. "Life would enter a new phase," says biophysicist Gregory Stock of UCLA, "one in which we seize control of our own evolution."

Judging by the 70 pages of public comments NIH has received since Anderson submitted his proposal in September, the overwhelming majority of scientists and ethicists weighing in oppose gene therapy that changes the "germline" (eggs and sperm). But the opposition could be a...
Parental Motivation

Risks to the Donor
Future
Gene Therapy
Will it work?
Creating Somatic Mosaicism

Chemotherapy?

NO Selective Growth Advantage

HSC

Selective Growth Advantage

Lessons of Nature
HSC Somatic Mosaicism
‘Natural Gene Therapy’

43 year old female
- Healthy
- MMC resistant
- Clonal hematopoiesis
- Double cross over event

FANCA gene mutations

Selective growth advantage
Risk of MDS/AL in residual FA cells

Lessons for FA Gene Therapy

IFAR 557/2

% Recovery

MMC Concentration

Normal Control

Revertant

FA Sibling Control

MMC resistant colonies

FISH with MLL probe exclusively found in colonies with FANCA mutations

Gregory JJ et al PNAS 2001; 98:2532
Rare Disease Transforms the Practice of Medicine

NMDP / Be the Match
UCB Banking and Transplantation
DNA Repair Genes
FA Cures
The Minnesota Family - 2009
Pediatric BMT

Bruce Blazar, M.D.
Christen Ebens, M.D.
Keli Hippen, Ph.D.
Margaret MacMillan, M.D.
Wes Miller, M.D.
Angela Mortari, Ph.D.
Paul Orchard, M.D.
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Adult BMT

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